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Site-specific mutagenesis of the reaction centre from *Rhodobacter sphaeroides* studied by Fourier transform Raman spectroscopy: mutations at tyrosine M210 do not affect the electronic structure of the primary donor

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Abstract

The effects of mutation of residue tyrosine M210 on the primary donor bacteriochlorophylls have been investigated by near infrared FT-Raman spectroscopy in reaction centres purified from an antenna-deficient strain of *Rhodobacter sphaeroides*. We find that mutation at the M210 position does not significantly perturb the distribution of the unpaired electron over the pair of bacteriochlorophyll molecules which constitute the primary donor radical cation. We conclude, therefore, that the effects of mutation of tyrosine M210 on the rate and asymmetry of primary electron transfer in reaction centres cannot be ascribed to a change in the electronic structure of the primary donor.

Key words: Reaction centre; Site-directed mutagenesis; FT-Raman; Tyrosine M210; Electron transfer; *Rhodobacter sphaeroides*

1. Introduction

The photochemical reaction centre is a membrane-bound protein complex which carries out light-driven vectoral electron transfer, generating a transmembrane electrical potential. The high resolution structures for this complex from the purple bacteria *Rhodopseudomonas viridis* [1] and *Rhodobacter sphaeroides* [2,3] have revealed the structural basis for the electron transfer reactions involved in the early stages of the photosynthetic process. Electron transfer is initiated by the arrival of a photon or excitation of appropriate energy at the primary donor (P) which is a dimer of Bchl molecules located near the periplasmic side of the membrane. The remaining pigments are arranged across the membrane dielectric in two approximately symmetrical branches, only one of which is active in mediating electron transfer (denoted the L branch). Photo-excitation of P into its first excited singlet state (P*) triggers the reduction of the Bphe on the active branch (H_L) in approximately 3 ps [4,5]. This is followed, sequentially, by the reduction of the quinone on the active branch (Q_A) in approximately 200 ps and reduction of the dissociable quinone on the inactive branch (Q_B) in a few microseconds (reviewed in [6]). The primary donor radical cation ($P^{+\bullet}$) is re-reduced, also on the timescale of a few microseconds, by an electron donated by, in *Rb. sphaeroides*, a soluble *c*-type cytochrome. In the course of reduction of H_L, the accessory Bchl on the active branch (B_L) may act as a distinct intermediate (through formation of the state $P^+B_L^-H_L$) or may mediate electron transfer through a superexchange mechanism (by participation in a virtual state) [7,8].

In recent years mutagenesis has been employed in order to gain insights into a number of aspects of reaction centre mechanism [9–11]. Of particular interest is the role of residue tyrosine M210, which lies on the apparent route of electron transfer from P to H_L and is in close contact with P, H_L and B_L [3]. The symmetry-related position on the inactive branch of the reaction centre is occupied by a phenylalanine residue (Phe L181) which is also highly conserved. It has been thought that tyrosine M210 may play a role in determining the rate of primary electron transfer and (in combination with Phe

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Abbreviations: Rb., Rhodobacter; RC, reaction centre; P, primary donor; $P^{+\bullet}$, primary donor radical cation; P*, primary donor first excited singlet state; Bchl, bacteriochlorophyll; Bphe, bacteriopheophytin; H_L, active branch acceptor Bphe; H_M, inactive branch Bphe; B_L, active branch accessory Bchl; B_M, inactive branch accessory Bchl; P_M and P_L, Bchls of the primary donor; Q_A, primary acceptor quinone; Q_B, secondary acceptor quinone; FT, Fourier transform; ENDOR, electron nuclear double resonance; LDAO, *N*,*N*-dimethyldodecylamine-*N*-oxide.

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L181) the asymmetry of the reaction. Previous mutagenesis studies in *Rb. sphaeroides* which targetted the M210 residue found that replacement of the tyrosine increased the time constant for the reduction of H_L from 3.5 ps to 16 ps and 22 ps for changes to Phe and Leu respectively [12], and to 10.5 ps and 16 ps for changes to Phe and Ile [13]. Recently it has also been reported that in reaction centres where the tyrosine at this position has been replaced by a phenylalanine, electron transfer to the Bphe on the inactive branch (H_M) can be observed under conditions where this does not occur in wild type centres [14], and experiments with mutants at the M210/L181 positions have also contributed to the proposal that during primary electron transfer the intermediate state $P^+B_M^-$ may be formed [15]. In the latter case the mutations appeared to modulate both the rate and extent of formation of this 'parking state' on the inactive branch. Hence the identity of the residues at the symmetry-related M210/L181 positions seems to exert considerable influence over the asymmetric nature of primary electron transfer.

Molecular orbital calculations have predicted that the electronic structure of the primary donor when in the P* and $P^{+\bullet}$ states may play a role in determining the asymmetry and efficiency of the charge separation reaction [16]. These calculations predict a surplus negative charge density on P_M (the primary donor Bchl hydrogen liganded to the M subunit) relative to P_L (the primary donor Bchl hydrogen liganded to the L subunit) which is enhanced by electrostatic interaction with the polar amino acid residues in the vicinity of P_M [17]. It should also be noted that a consideration of the nearest atom distances (excluding hydrogens) between P_L, P_M and B_L reveals that the distance between B_L and P_M is in fact shorter than the distance between B_L and P_L [17]. Hence a combination of the electronic and structural asymmetry of P enhances the electronic coupling of P_M* with B_L and may be a major factor in determining the route of electron transfer down the active branch. Following the formation of the $P^+H_L^-$ state, the localisation of the electron hole on P_L would help to impede the back reaction from H_L^- [16]. Recent measurements by near-infrared Fourier transform Raman (FT-Raman) spectroscopy [18,19] and by electron nuclear double resonance (ENDOR) [20] have indeed revealed an asymmetric distribution of charge over the two Bchls of the primary donor, with the larger part of the positive charge being carried by P_L.

Given that mutation at the M210 position appears to sizeably affect the rate of primary charge separation and may also influence the balance of electron flow down the active and inactive branches, it was of interest to see whether mutations at this position affected the electronic structure of the primary donor. Accordingly we have applied FT-Raman spectroscopy to reaction centres of *Rb. sphaeroides* bearing mutations at the M210 position in order to investigate the environment of P in the ground state and the structure of $P^{+\bullet}$.

2. Experimental

2.1. Media, strains, plasmids and growth conditions

Bacterial strains and plasmids are as described in [21]. *Escherichia coli* strains were grown in Luria Broth with appropriate antibiotics. *Rb. sphaeroides* strains were grown under semi-aerobic/dark conditions in M22+ medium [22] supplemented with 0.1% casamino acids for growth in liquid culture. All *Rb. sphaeroides* strains were grown in the presence of streptomycin and kanamycin; for the growth of strains which had been complemented with reaction centre genes, tetracycline was also included. Antibiotic concentrations were as described in [21].

2.2. Construction of the site-directed mutant strains and purification of the mutant reaction centres

Site-directed mutagenesis of the M subunit of the reaction centre was carried out using an approximately 1.0-kb *SalI–Bam*HI restriction fragment encompassing the *pufM* gene excised from plasmid pRKEH10D [21] and subcloned into the multiple cloning site of bacteriophage M13mp19. Oligonucleotide-mediated mutagenesis was performed according to the method of Kunkel [23]. Site-directed changes were restricted to the codon for the target residue tyrosine M210 and were TAC→TTC (Tyr→Phe), TAC→CAC (Tyr→His) and TAC→CTA (Tyr→Leu). Mutagenised sequences were identified by sequencing using the dideoxy chain termination method of Sanger et al. [24]. Mutant sequences were transferred to plasmid pRKEH10D in place of the wild type sequence prior to conjugation into *Rb. sphaeroides*.

Plasmids to be introduced into the *Rb. sphaeroides* deletion strains were first transformed into *E. coli* strain S17–1. Matings were performed as described in [22] using as host the double deletion mutant strain DD13/G1 [25]. Selection was for dark/aerobic growth on plates of M22+ medium supplemented with tetracycline and kanamycin. The presence of reaction centres in the transconjugant strains was confirmed by absorption spectroscopy of the bacterial colonies using a Guided Wave model 260 fibre optic spectrophotometer. None of the site-directed mutations introduced into the *pufM* gene at the M210 position appeared to have a detrimental effect on the steady state number of reaction centres per cell, with the level of reaction centre expression being similar to that previously determined for strain RCO1 [21] which was used as the control strain in this study.

2.3. Growth of cells, preparation of membranes and isolation of reaction centres

All *Rb. sphaeroides* strains bearing mutant *pufM* genes were grown under dark/semi-aerobic conditions in the presence of streptomycin, kanamycin and tetracycline. These conditions minimise the possiblity of reversion of the site-directed mutation. As a further safeguard the sequence of the M subunit was checked in DNA prepared from cells that had been used to provide membranes. This was done by isolation of the plasmid DNA from an aliquot of cells using the alkaline lysis method followed by transformation of the DNA into *E. coli* for amplification. The *pufM* gene was then subcloned into either bacteriophage M13mp19 or pBluescriptKS+ for sequencing.

Cells were harvested by centrifugation and washed once with 10 mM Tris (pH 8.0). Harvested cells were resuspended to 0.5 g wet weight ml^{-1} in 10 mM Tris (pH 8.0) and broken in a French Pressure Cell (Aminco). Membranes were purified by ultracentrifugation at 4°C and 27,000 rpm in a TY45 rotor (Beckman) for a minimum of 4 h using a sucrose step gradient (15–40%, wt/wt). Membranes removed from the interface of the step gradient were concentrated by dilution in 10 mM Tris (pH 8.0) followed by ultracentrifugation for 2 h at 4°C and 40,000 rpm also in a TY45 rotor. RC-only membranes prepared in this way were stored as pellets at −20°C prior to use.

Purification of wild type or mutant reaction centres was carried out in dim light at 4°C. Frozen RC-only membranes were thawed and diluted in 25 mM Tris (pH 8.0) to an A_{800} of 8.0. This suspension was treated with 0.3% LDAO for 5 min at room temperature and centrifuged at 45,000 rpm in a Ti 50 rotor (Beckman) for 1 h. The supernatant containing solubilised reaction centres was loaded onto a DEAE 52

column which had been equilibrated with 25 mM Tris/0.1% LDAO (pH 8.0) and submitted to a series of washes with 25 mM Tris (pH 8.0) containing an increasing concentration of NaCl. When the column was carefully equilibrated between each (20 mM) step of the gradient, reaction centres generally eluted at between 120 and 180 mM NaCl. Eluted reaction centres were concentrated to an A_{800} of 1.0 using Centricon microconcentrators (Amicon) and the LDAO detergent exchanged for 0.1% cholate by overnight dialysis against 25 mM Tris/0.1% cholate (pH 8.0).

2.4. Absorbance and FT-Raman spectroscopy

Absorbance measurements were made using a Cary 2300 scanning spectrophotometer. Samples were suspended in 60% glycerol and the temperature was regulated at approximately 30 K using a gas flow cryostat cooled with cold helium gas. For FT-Raman purified reaction centres were concentrated using a Centricon 100 semipermeable membrane (Amicon) to give an A_{800} of between 50 and 150. Measurements were performed at room temperature with a Bruker IFS 66 interferometer coupled to a Bruker FRA 106 Raman module equipped with a continuous diode-pumped Nd:YAG laser. Experimental procedures were essentially as described in [19]

3. Results and discussion

3.1. Low temperature absorption spectroscopy and resonance Raman of purified mutant reaction centres

In previous reports, Gray and co-workers have described purified reaction centres bearing mutations at the M210 position which showed evidence of having undergone changes in their global structure [26,27]. These were manifested in the loss of the normally integral quinone from the Q_A site [26] and alterations in the absorbance and resonance Raman properties of H_M [27]. As such global changes could be expected to perturb the electronic and electron transfer properties of the reaction centre in a non-specific manner, we have used low temperature absorption spectroscopy and UV Raman spectroscopy to assess the overall structural integrity of the mutant reaction centres following purification from RC-only membranes using the protocol described above.

Spectra of the Q_x and Q_y transitions of the Bchls and Bphes were recorded at low temperature (30 K) for untreated purified reaction centres (Fig. 1). The band attributed to the low-energy exciton component of the Q_y transition of the primary donor, located at 893 nm in the spectrum of wild type reaction centres, was shifted slightly to the red (by between 3 and 4 nm) in the spectra of the mutant reaction centres (Fig. 1B). In all three mutant reaction centres this band could be fully bleached either by chemical oxidation using ferricyanide or by illumination at room temperature under saturating light conditions.

The band at 802 nm in the spectrum of wild type reaction centres is a composite of the Q_y transitions of the monomeric Bchls (B_L and B_M) and the weak upper-energy exciton component of the Q_y transition of the primary donor. This band was found to be red-shifted to between 805 and 807 nm in the mutants and showed a relative increase in intensity, alterations which were also observed by Gray et al. [26] in mutant reaction centres

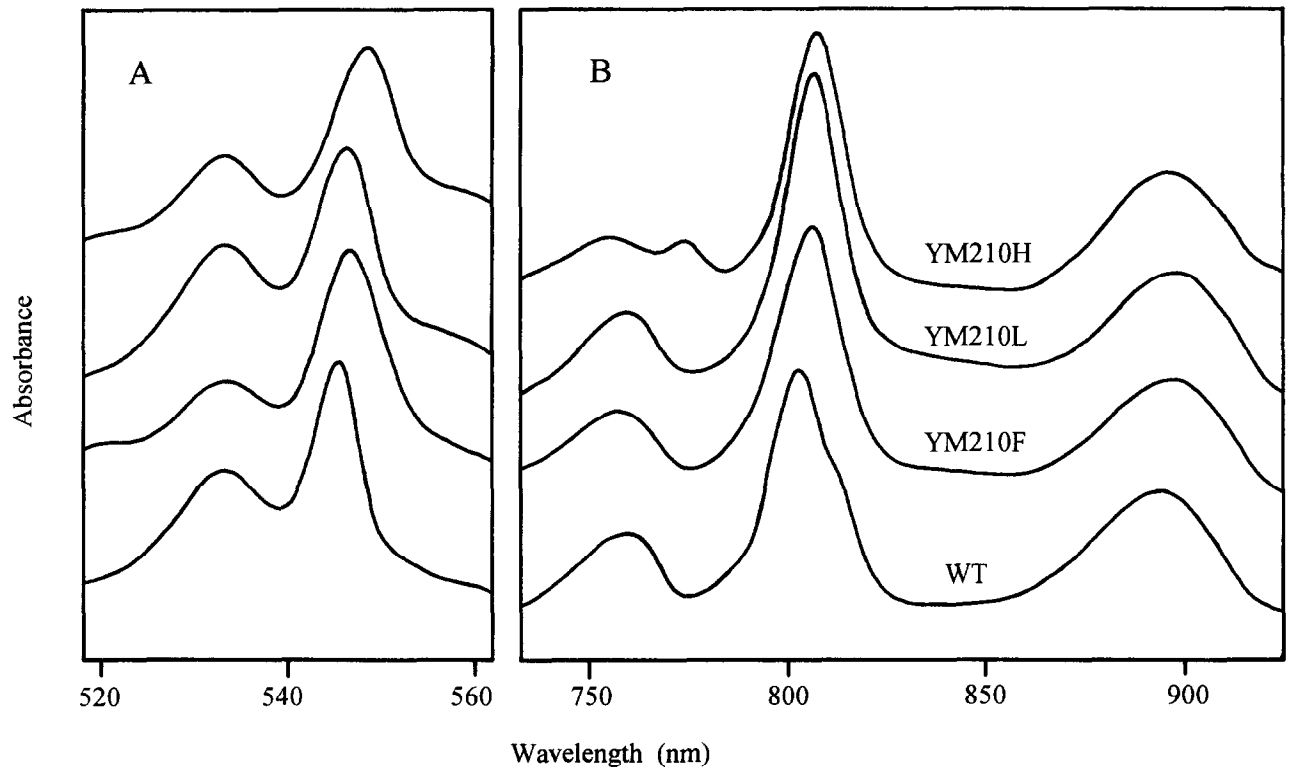

Fig. 1. 30 K absorption spectra of (A) the Q_x bands of H_L and H_M and (B) the Q_y bands of all six bacteriochlorins of the reaction centre. Wild type and mutant reaction centres were purified and suspended in 60% glycerol. Absorbance is shown on an arbitrary scale. For comparison, spectra were normalised to the height of the major absorption band and offset.

with a phenylalanine or leucine residue at the M210 position. The increase in intensity of this band was considered to arise either as a consequence of the better spectral overlap between the Q_y transitions of B_L and B_M following a shift of the B_L transition to a lower energy and/or as a result of an increase in the oscillator strength of the B_L transition [26]. We have computed difference spectra (mutant minus wild type) in this wavelength region using the low-energy exciton component of P as a reference band for normalisation (data not shown). Assuming that the intensities of the other two components of the 807 nm band do not change as a result of mutation, the results indicate that the oscillator strength of the Q_y transition of B_L is increased in intensity by approximately 10% relative to the wild type.

The band in the spectrum of wild type reaction centres at 759 nm which is attributed to the Q_y transition of the Bphes was located at 757 nm in the Tyr M210→Phe and 759 nm in the Tyr M210→Leu mutant. In reaction centres from the Tyr M210→His mutant this band split into two components, centred at approximately 754 nm and 774 nm, suggesting a red-shift in the position of the band associated with the Q_y transition of one of the Bphes. This phenomenon has previously been described for reaction centres from a mutant of *Rhodobacter capsulatus* bearing the change Tyr M210→His [28] and may indicate the formation of a hydrogen bond to the 2-acetyl carbonyl group of H_L. We are presently conducting resonance Raman experiments to test this hypothesis.

At room temperature the Q_x transitions of the active- and inactive branch Bphes (H_L and H_M, respectively) give rise to a single absorbance peak at approximately 540 nm. At low temperature two discrete peaks can be resolved at approximately 545 nm (for the Q_x of H_L) and 535 nm (for the Q_x of H_M). The purified mutant reaction centres described in [26] which bore the change Tyr M210→Phe and Tyr M210→Leu both exhibited a red-shift in the position of the 535 nm band of 2–3 nm, considerably increasing the overlap between the H_L and H_M Q_x peaks [14]. However, in all cases our purified mutant reaction centres showed no alteration in the position of the H_M Q_x band relative to its position in the spectrum of the wild type, with well resolved peaks being observed at 533 nm and 545 nm for the wild type and at 533 and 546 nm in the Tyr M210→Leu and Tyr M210→Phe mutants. In the Tyr M210→His mutant, the Q_x band of H_L was further red-shifted by 2 nm to 548 nm. This correlates with the observation, described above, that reaction centres from our Tyr M210→His mutant also show a relative decrease in the energy of the Q_y transition of one of the Bphes, resulting in a splitting of the band normally centred at 757 nm.

Changes in the structure of the protein in the vicinity of H_M in the mutant complexes described in [26] were also detected by resonance Raman employing excitation at 363.8 nm [27]. These changes were evidenced by a marked weakening (leucine) or loss (phenylalanine) of a band at 1703 cm^{-1} which has been ascribed to the stretching frequency of the free keto carbonyl group of H_M [29]. We therefore have examined our mutant centres by UV-Raman (data not shown) and found no significant differences between spectra of the mutants and that of the wild type complex, which in turn gave a spectrum in full accord with that published previously for the *Rb. sphaeroides* reaction centre [30]. In particular the band at 1703 cm^{-1} was clearly present in spectra for all three mutant complexes.

3.2 *Integrity of the primary acceptor quinone (Q_A) in purified mutant centres*

The reaction centre contains two quinone molecules, one of which (Q_A) is tightly bound and acts as a one electron carrier and the other of which (Q_B) can dissociate from its binding site into the membrane phase and acts as a two electron\two proton carrier. Purification of the reaction centre usually results in the loss of a proportion of the Q_B quinone, but the integral Q_A quinone is retained. The mutant reaction centres described in [26] were unusual in that they showed a marked (85% to 95%) loss of the Q_A quinone (which could be partially reconstituted). In the light of this we have investigated the occupancy of the Q_A site by examining the ability of our purified mutant reaction centres to undergo reversible photooxidation. This was done by monitoring the effect of saturating light on the intensity of the peak attributed to the low-energy exciton component of the primary donor (at 865 nm at room temperature) (data not shown). In all three mutants this peak was bleached by greater than 90% upon illumination, indicating a greater than 90% occupancy of the Q_A site in our purified mutant centres. Consideration of the rate of decay of this bleaching after switching off the actinic light further suggested that approximately 30% of the Q_B sites were occupied in our mutant centres (not shown).

Taken together, the low temperature absorbance and UV-Raman data presented in the last two sections indicate that our mutant centres were not altered in terms of global structure, but did show minor alterations in the vicinity of the mutated residue i.e. red-shifts of 3 to 5 nm in the position of the Q_y bands for B_L and P. These observations are important, as most interpretations of the results of time-resolved or steady state measurements on mutant reaction centres start from the assumption that the global structure of the complex has not been altered by the mutation. In the present study the most marked changes were observed in the case of the change Tyr M210→His where red-shifts were observed in the position of the Bphe Q_y and Q_x peaks. This finding is in accord with results from site-directed mutants of the *Rb. capsulatus* reaction centre [28] and suggests an interaction between the histidine at the M210 position and H_L.

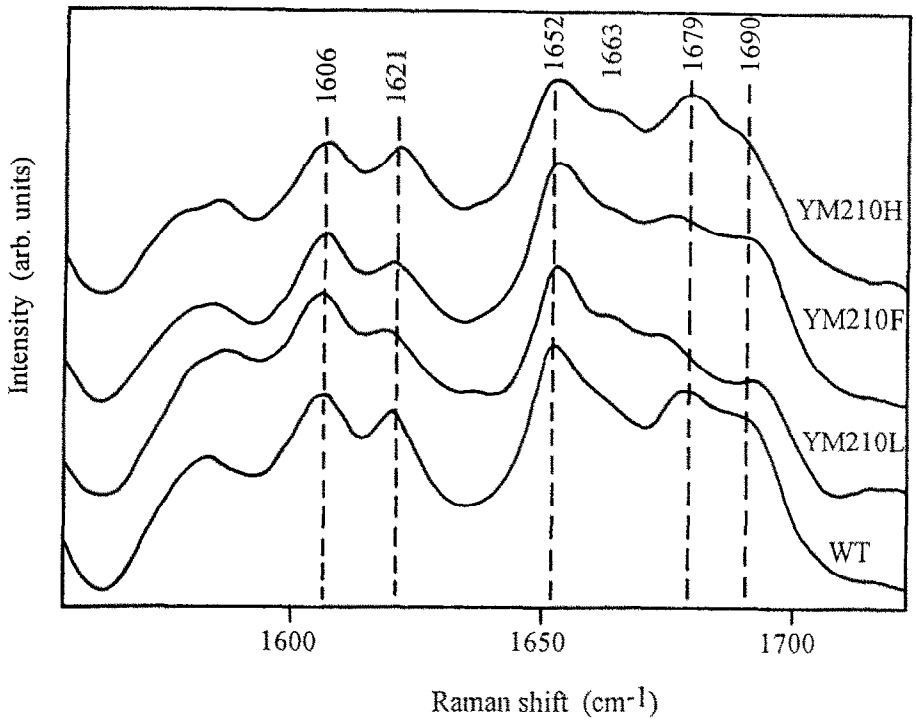

Fig. 2. FT-Raman spectra of wild type and mutant reaction centres with the primary donor in the neutral state. Accumulation of reaction centres in the photo-oxidised state was prevented by the addition of ascorbate. The spectra display that part of the high frequency region which contains bands attributable to the 2-acetyl and 9-keto carbonyl groups of the primary donor.

3.3. Integrity of the primary donor in mutant reaction centres assessed by FT-Raman spectroscopy

In contrast to UV Raman, which yields spectra which contain information on all six bacteriochlorin pigments, FT-Raman spectra contain vibrational information which is very largely specific to the primary donor [18,19]. The spectrum of P in the neutral state may be obtained by FT-Raman without the need to resort to

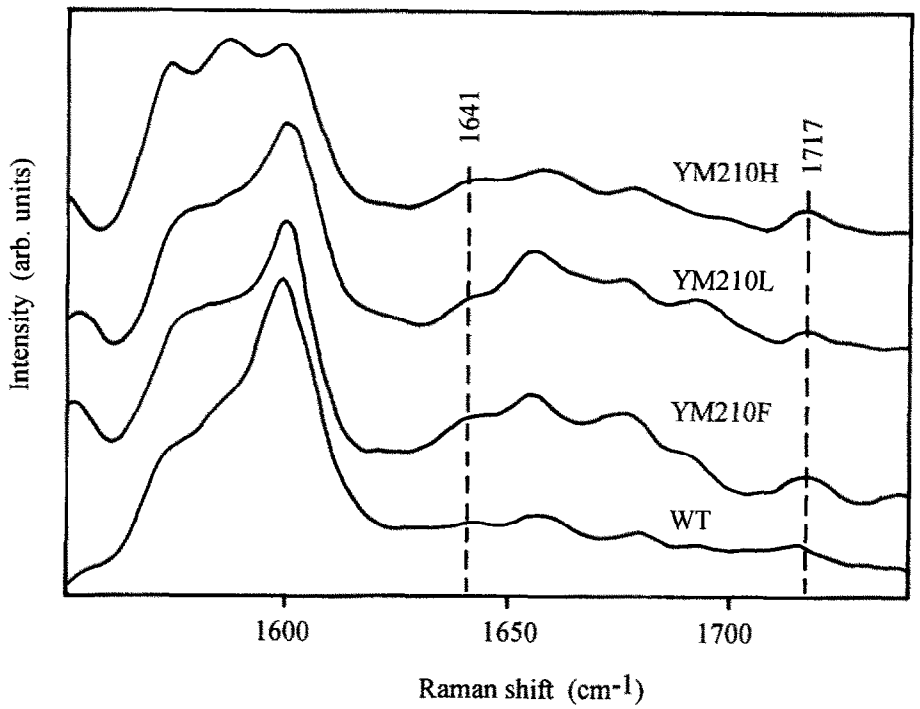

Fig. 3. FT-Raman spectra of wild type and mutant reaction centres with the primary donor in the radical cation state $P^{+\bullet}$. Raman spectra of purified reaction centres were recorded in the absence of ascorbate, resulting in the photo-oxidation of the primary donor through the actinic effect of the laser.

difference techniques, and therefore is characterised by a very good signal to noise ratio. This allows the position of the bands in the spectrum to be determined with a higher degree of precision than has been the case in previous studies employing Soret excitation to study mutants at the M210 position [27]. Before examining the distribution of charge over the primary donor we first examined the FT-Raman spectra of the mutant complexes in the neutral state. This is a prerequisite as the distribution of charge over the primary donor in the $P^{+\bullet}$ state is assessed from an upshift in the position of the bands attributable to the stretching frequency of the 2-acetyl and 9-keto carbonyl groups of P_L and hence it is necessary to know the position of these bands in the ground state spectrum. The position and intensities of the bands in the FT-Raman spectrum of the neutral state also gives information on the environment of the primary donor. In order to prevent formation of the radical cation $P^{+\bullet}$ due to light emitted by the diode-pumped 1064 nm laser, ascorbate was added to the samples to maintain the primary donor in the neutral state.

Fig. 2 displays the high frequency region of the FT-Raman spectra of wild type reaction centres and those purified from our three site-directed mutants. The frequencies of the four bands contributed by the 2-acetyl and 9-keto carbonyl groups of P have been characterised for wild type reaction centres of *Rb. sphaeroides* in a previous study and were reported to be 1620 cm^{-1} (2-acetyl of P_L), 1653 cm^{-1} (2-acetyl of P_M), 1679 cm^{-1} (9-keto of P_M) and 1691 cm^{-1} (9-keto of P_L) [31]. As displayed in Fig. 2, wild type reaction centres extracted from strain RCO1 exhibit, within a certainty of approximately $\pm$ 1 cm^{-1}, the same FT-Raman spectrum as has been published previously for reaction centres isolated from wild type strains of *Rb. sphaeroides*, with bands at 1621, 1652, 1679 and 1690 cm^{-1}. An additional band at 1663 cm^{-1} can also be observed under the present conditions of excitation but, as this band does not bleach upon photooxidation of the primary donor, it has been attributed either to the accessory Bchls or to protein features [31]. The band at 1606 cm^{-1} is due to the C_aC_m stretching mode and its position and narrow bandwidth (ca. 14 cm^{-1}) indicates that both P_L and P_M possess a single axial ligand to the central magnesium atom. In reaction centres from the Tyr M210 $\rightarrow$ His mutant none of the frequencies of the carbonyl groups was shifted by more than 2 cm^{-1} relative to those of the wild type, indicating no change in the pattern of hydrogen bonds to the primary donor, nor any sizeable change in the strength of these H-bonds. In reaction centres from the Tyr M210 $\rightarrow$ Phe and Tyr M210 $\rightarrow$ Leu mutants the FT-Raman spectra exhibit the same frequencies as those seen in wild type reaction centres with the exception of the 1679 cm^{-1} band which is clearly shifted to 1676 cm^{-1} in the Phe mutant and 1674 cm^{-1} in the Leu mutant. The fact that only one band is affected by the series of mutations strongly suggests that this shift is not due to a destabilization of the reaction centre structure, but rather to a specific effect due to the actual mutation. The shift could arise from a change in the dielectric constant local to this carbonyl on mutation to a non-polar residue such as phenylalanine or leucine, but it is too small to indicate the formation or breakage of a hydrogen bond (which would be expected to produce a shift of between 20 and 50 cm^{-1}). We are currently modifying our FT-Raman instrument in order to record spectra of this sort with reaction centres which are still embedded in the intracytoplasmic membrane, in order to ascertain whether this small alteration of the spectrum is still present in membrane-bound complexes.

3.4. *The effect of mutation at the M210 position on the distribution of charge over* $P^{+\bullet}$

Fig. 3 displays the FT-Raman spectra of reaction centres from the control and mutant RC-only strains recorded in the absence of ascorbate which, as described above, are expected to contain prominent contributions arising from $P^{+\bullet}$. In the carbonyl stretching region, the spectra of wild type reaction centres from *Rb. sphaeroides* contain the non-bleachable band at 1663 cm^{-1} described above, small bands at frequencies which match those observed in ascorbate-treated reaction centres and which arise from a minor population of reaction centres in the neutral state, and two new bands at 1641 and 1717 cm^{-1}. The latter have been attributed to the 2-acetyl and 9-keto carbonyl stretching modes of P_L when in the radical cation state, and their properties have been discussed in detail in a previous paper [19]. In vitro studies have shown that, upon oxidation of Bchl in solution, an upshift of both the 9-keto and 2-acetyl carbonyl stretching frequencies is observed [32]. By comparing the extent of this upshift for a Bchl cation with that observed for the P_L Bchl of the primary donor, where the charge is to some extent shared between P_L and P_M, it is possible to evaluate the proportion of the charge of the cation that is localised on the P_L molecule. As the keto carbonyl group of P_L is expected to assume little distortion from its normal in-plane conformation, the stretching mode of this group was chosen for this evaluation. In experiments with free Bchl *a* in an apolar solvent, this 9-keto mode, which is free from interactions in an apolar environment, undergoes an upshift of 32 cm^{-1} on transition from the neutral state to the cation. In reaction centres from wild type strains of *Rb. sphaeroides* this stretching mode is observed at 1691 cm^{-1} when the primary donor is in the neutral state and 1717 cm^{-1} when in the cationic state, an upshift on photooxidation of 26 cm^{-1} [19]. This has been taken as direct evidence that the P_L molecule carries most of the photoinduced positive charge that is distributed over the primary donor [19]. A very precise evaluation of the fraction of the positive charge carried by the P_L molecule cannot be determined due to the differences

between the environment of Bchl molecule in vivo and in vitro. However, a reasonable estimate is that 80 ± 10% of the positive charge is carried on P_L.

Although absolute values for the extent of charge localisation on P_L cannot be precisely calculated, a comparative study can yield very accurate information on the effect of mutation on the distribution of charge over P. As noted above, the position of the band attributed to the stretching frequency of the 9-keto carbonyl of P_L in the neutral state is highly conserved between the wild type and three site directed mutants examined in this study (within ± 1 cm^{-1}; Fig. 2). As shown in Fig. 3 the position of the upshifted carbonyl frequency in experiments with oxidised reaction centres was also conserved to within ± 1 cm^{-1}, being located at approximately 1717 cm^{-1} in spectra of the wild type and all three mutant reaction centres. In Fig. 3 the 1550–1600 cm^{-1} region of the FT-Raman spectrum of the Tyr M210 → His mutant reaction centres is sizeably different from that in the spectra of the wild type and remaining mutants. This effect may be due to changes in the absorption of the primary donor radical cation and is currently under investigation. From these experiments we can conclude that no change occurs in the distribution of charge between the Bchls of the primary donor upon mutation of the residue at the M210 position to Phe, His or Leu.

3.5. Conclusions

An intriguing feature of the reaction centre is the marked asymmetry of primary electron transfer. It is now emerging that this phenomenon is at least in part determined by an asymmetry in the electronic structure of the primary donor which favours electron flow down the active branch. There is increasing evidence that the residue at the M210 position plays a role in determining both the rate of charge separation and the balance of electron flow down the active and inactive branches. From the data presented in this report we conclude that these effects do not stem from a significant change in either the physical or electronic structure of the primary donor.

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